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SHELL ULTRASTRUCTURE IN TWO SUBSPECIES OF THE RIBBED MUSSEL, *GEUKENSIA DEMISSA* (DILLWYN, 1817)¹

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Geukensia demissa (= *Modiolus demissus*), the ribbed mussel, is a common infaunal constituent of salt marshes on all coasts of North America. There are two subspecies. The northern one, *G. d. demissa* (Dillwyn, 1817), ranges from the Gulf of St. Lawrence to Florida (Abbott, 1974); it is also present on the west coast, having been introduced to San Francisco Bay (Hanna, 1966). The southern subspecies, *G. d. granosissima* (Sowerby, 1914), is found on both coasts of Florida and along the gulf coast to Yucatan (Andrews, 1971). The ranges overlap along the east coast of Florida, between Duval and Palm Beach Counties (unpublished collections of M. J. and P. A. Greenberg, and D. A. Price).

The two subspecies are distinguished on the basis of differences in gross shell morphology (Sowerby, 1914; Andrews, 1971; Abbott, 1974). This paper describes differences in the coloration, growth surface and ultrastructure of the shells of *G. d. demissa* and *G. d. granosissima* and suggests that these differences are characteristic of the subspecies and not of the climatic gradient coinciding with the geographical range of the species.

In 1955, Soot-Ryen assigned the common ribbed mussel, *Modiolus demissus*, to the genus *Arcuatula* on his interpretation of Jousseaume's description in Lamy (1919). Van de Poel (1959) noted that the interpretation was in error and introduced a new genus name, *Geukensia* (in honor of Professor F. Geukens of the University of Louvain). Soot-Ryen evidently concurred, for in his revision of the superfamily Mytilacea in the *Treatise on Invertebrate Paleontology* (1969), he referred the Atlantic ribbed mussel [*Modiola plicatula* Lamareck, 1819 (= *Modiola demissa* Dillwyn, 1817)] to the genus *Geukensia* Poel, 1959 as the type species, now *G. demissa* (Dillwyn, 1817).

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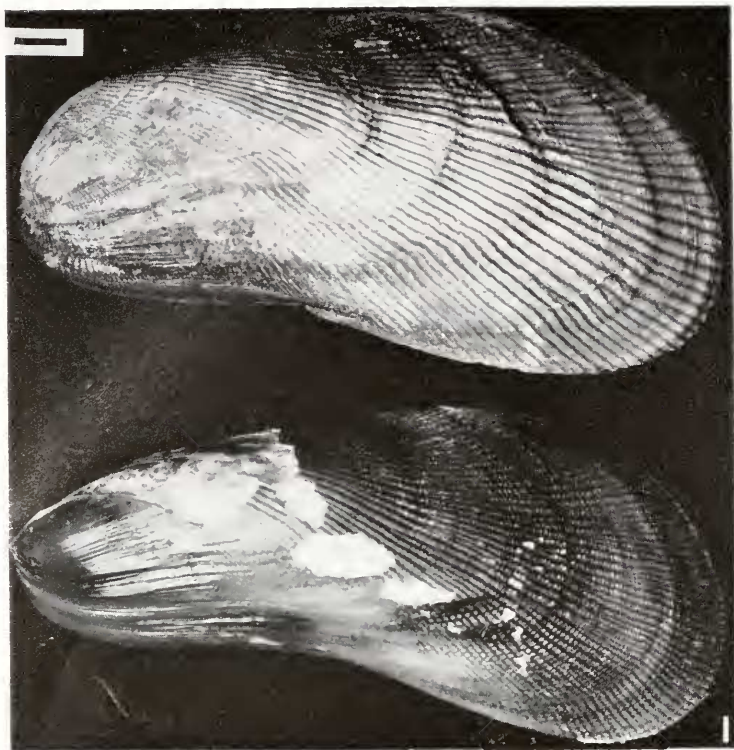


FIGURE 1. Shells of *Geukensia d. demissa* (top) with smooth wide ribs and *G. d. granosissima* (bottom) with narrow granulated ribs. Scale bar is 5 mm.

Recent manuals have been employing *Geukensia* as the name of the genus (e.g., Andrews, 1971; Abbott, 1974). Although Keen and Coan (1974) have made *Geukensia* a subgenus of *Ischadium*, following the analysis and recommendations of Kenk (1966), the hard- and soft-part morphological and the physiological differences discussed by Pierce (1973) suggest that *Geukensia* should be a separate genus.

MATERIALS AND METHODS

Specimens of *Geukensia demissa demissa*, 7 to 9 cm long, were collected from salt marshes in Oyster Creek, Maine; Oyster Bay, Long Island, New York; Sippewissett Marsh on Cape Cod, Massachusetts; Sapelo Island, Georgia; and Duval and Palm Beach Counties in Florida. Specimens of *G. d. granosissima*, 6 to 8 cm long, were collected in Florida from Duval, Volusia, Franklin and Sarasota counties. The soft parts were removed and the shells retained for sectioning.

Three kinds of observations were made. To examine the growth surface, the shells were cut parallel to the ribs, rinsed in distilled water, then bleached in 5.25% sodium hypochlorite (Clorox) for 1.5 to 2 hours to loosen the periostracum. The periostracum was then gently removed with forceps. To examine the shell

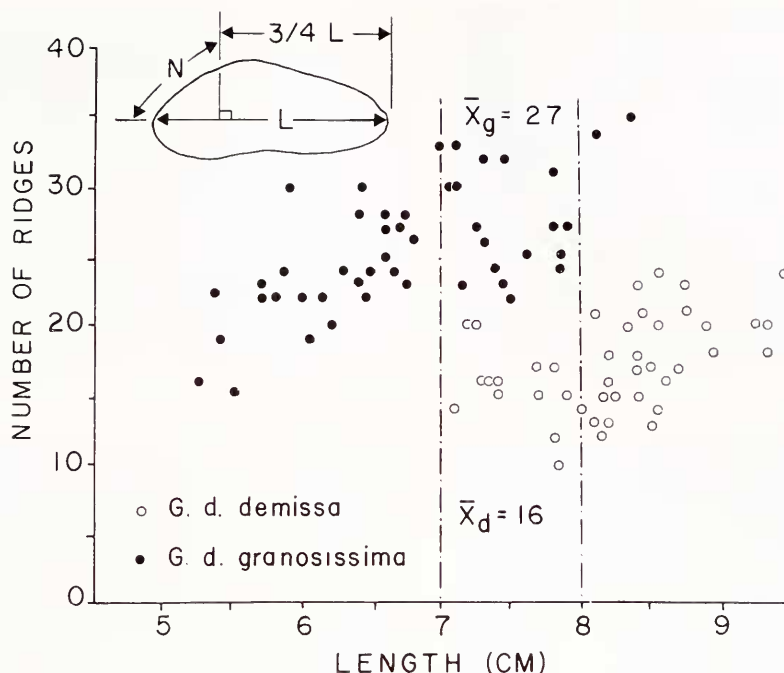


FIGURE 2. Relationship between the number of ribs and the size of the shell in the two subspecies. \bar{X}_a , \bar{X}_g indicate the mean number of ribs in shells (*Geukensia d. demissa* and *G. d. granosissima*, respectively) between 7 and 8 cm long. The method of estimating the number of ribs (inset) was: first, at a point $3/4$ of the distance from the anterior end of the shell, along the longitudinal axis ($3/4 L$), a line was erected perpendicular to the axis; then, the number (N) of ribs was counted along the edge subtended by the 90° angle.

layers, sections were cut parallel and perpendicular to the ribs, polished on a lap wheel, cleaned in an ultrasonic cleaner for two minutes with several washes, etched in 1% hydrochloric acid for fifteen seconds, and then bleached for 30–45 minutes. To differentiate between calcite and aragonite, etched polished sections were stained with Feigl's solution as modified by Schneidermann and Sandberg (1971). All shell sections were cut on a diamond saw.



FIGURE 3. *Geukensia d. demissa*: a Feigl's stained section cut parallel to the ribs showing the outer unstained calcite (UC) and the inner stained aragonite (SA). Scale bar is 100 μ m.

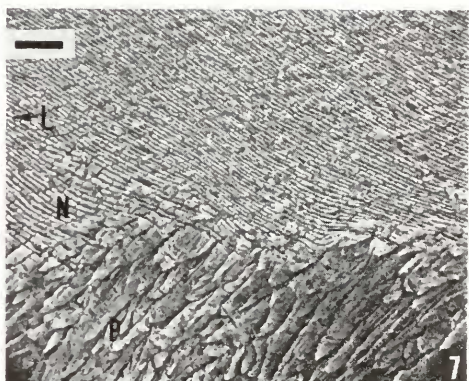
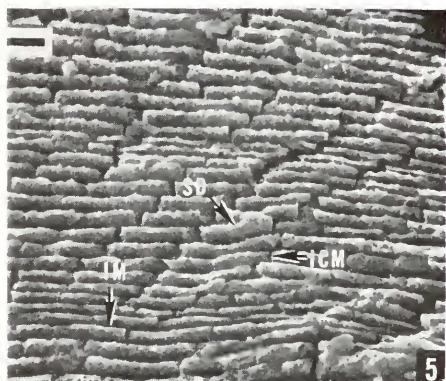
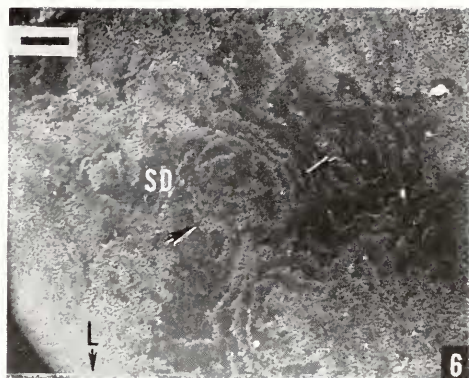
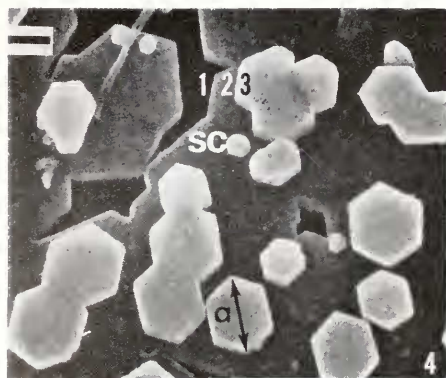


FIGURE 4. Growth surface of *Geukensia d. demissa* showing three developing layers (1, 2, 3). The hexagonal crystals are aligned with their a-axes (a) approximately in parallel. SC represents seed crystal; scale bar is 2 μm .

FIGURE 5. *Geukensia d. demissa*: polished section cut parallel to ribs showing the treppen (staircase) arrangement of the crystals. IM represents interlamellar matrix; ICM, intercrystalline matrix; SD, screw dislocation; scale bar is 2 μm .

FIGURE 6. Growth surface of *Geukensia d. demissa* showing a screw dislocation in the nacre (bracketed by arrows). L indicates direction toward lip of shell; scale bar is 43 μm .

FIGURE 7. *Geukensia d. demissa*: polished section cut parallel to ribs. The orientation of the prisms (P) to the nacre (N) is regular. Compare with Figure 8. L indicates direction toward lip of shell; scale bar is 8 μm .

Prepared sections were glued to stubs and coated with gold palladium in a vacuum evaporator (Denton DV502). The shells were examined with a scanning electron microscope (Cambridge Stereoscan Mark 2-A or S4-10).

To contrast the difference in coloration the shells were bleached eight to ten hours in 5.25% sodium hypochlorite (Clorox); this treatment removed all of the periostracum.

RESULTS

The gross shell morphologies of *G. d. demissa* and *G. d. granosissima* are easily distinguished (Sowerby, 1914; Abbott, 1974). In specimens of the same size, *G. d. granosissima* shells have numerous, narrow, granulated (or beaded) ribs; the ribs of *G. d. demissa* are fewer in number, wider and smooth (Fig. 1). The difference in rib number is immediately apparent (Fig. 1) and easily quantified (Fig. 2).

The shell of both subspecies consists of a thick calcified portion overlaid by a thin outer organic periostracum. The calcified part of the shell is divided into three layers: innermost is a nacreous layer (approximately 1 mm thick) comprising the major portion of the shell; a middle prismatic layer (0.1 mm thick) follows; and a thin (1 μ m) prismatic layer is outermost. Both the nacreous and middle prismatic layers stain with Feigl's solution and are aragonite (Fig. 3). The thin outer layer does not stain and is calcite.

The nacreous layer

The nacreous layer is composed of laminae of contiguous hexagonal crystals (Fig. 4). Occasionally, rounded crystals were observed; these probably formed during periods of rapid growth (Wada, 1961). The intercrystalline space, 50–60 nm wide, contains the intercrystalline matrix in unbleached sections (Fig. 5). The interlamellar space, 100–150 nm thick, is occupied in unbleached specimens by the interlamellar matrix (Fig. 5). The crystals are elongated to varying degrees along the crystallographic *a*-axis and are oriented with their *a*-axes parallel to each other (Fig. 4) and to the inner surface of the shell. In cross section, the arrangement corresponds to the "treppen pattern" (Fig. 5) described by Schmidt (1923).

Screw dislocations were observed on the growth surface (Fig. 6) and also in polished sections (Fig. 5) on both subspecies. In Figure 4, small seed crystals are seen on, or close to, the edges of the parent crystal on the 001 face, indicating that crystal formation may be occurring by the mechanism of screw dislocation as proposed by Taylor, Kennedy and Hall (1969).

The middle prismatic layer

The middle prismatic layer in both subspecies is composed of conical aragonite prisms, the cones measuring 10 μ m at their bases and 20–30 μ m in height. The

FIGURE 8. *Geukensia d. granosissima*: polished section cut parallel to the ribs. The orientation of the aragonite prisms (P) varies from 0° to 90° in a granule. The arrow indicates the anterior end of a granule. L indicates direction toward lip of shell; o represents outer surface of the shell; scale bar is 40 μ m.

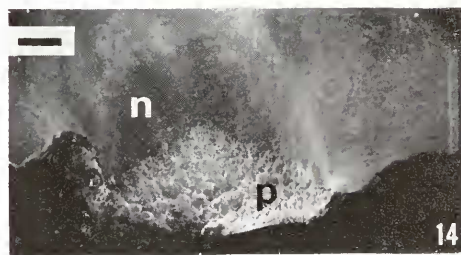
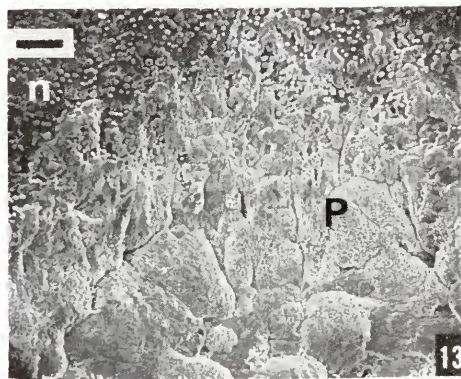
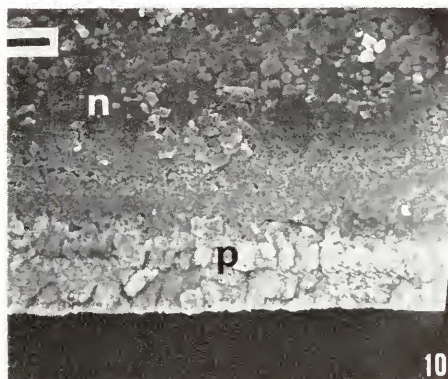
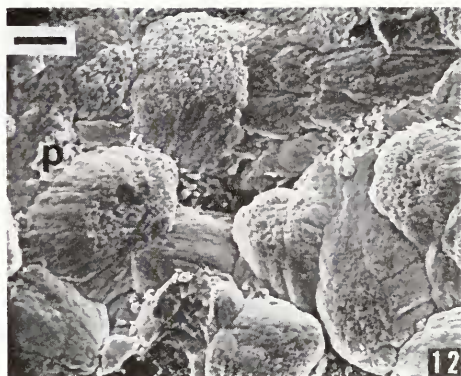
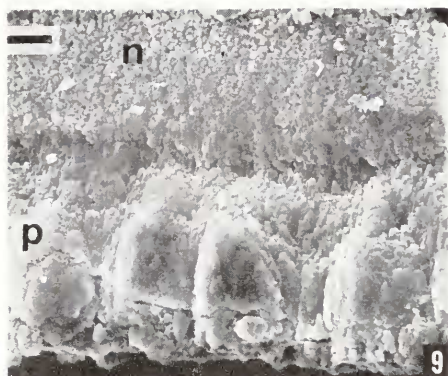


FIGURE 9. *Geukensia d. demissa*: enlargement of Figure 10 showing conical prisms in the prismatic layer (p); n represents nacre; scale bar is 10 μ m.

FIGURE 10. Enlargement of Figure 11 showing regular interface between nacre (n) and prismatic (p) layers. Compare with Figure 13. Scale bar is 18 μ m.

FIGURE 11. Growth surface of *Geukensia d. demissa* showing the prismatic layer (p) along the shell edge. Compare with Figure 14; n represents nacre. Scale bar is 100 μ m.

FIGURE 12. *Geukensia d. granosissima*: enlargement of Figure 13 showing irregular arrangement of conical prisms in the prismatic layer (p) on the underside of a granulated rib. Scale bar is 10 μ m.

FIGURE 13. *Geukensia d. granosissima*: enlargement of Figure 14. The interface between the nacreous (n) and prismatic layers (p) is curved along the posterior edge of the granule. Scale bar is 18 μ m.

arrangement of the prisms is similar to the flabellate structure found in some mytilids (Oberling, 1964).

The bulk of each of the conspicuous granules or beads on the ribs of *G. d. granosissima* is composed of prismatic aragonite. In the development of each granule, the prisms are deposited in a fanned array such that the angle between the columns of prisms and the nacreous-prismatic interface varies from nearly 0° to almost 90° (Fig. 8) from one side of the granule to the other. In *G. d. demissa*, which has smooth ribs, the arrangement of aragonite cones is more regular (Figs. 7, 9, 10, 11). The difference is evident in polished sections (compare Fig. 7 and Fig. 8) and in sections of the growth surface (compare Figs. 9, 10, and 11 to Figs. 12, 13, and 14). In *G. d. demissa*, the prisms are perpendicular to the nacre in the plane of the ribs, and the angle is constant (Fig. 7).

Along the growth surface in *G. d. demissa*, the prismatic aragonite forms a layer of uniform thickness across the lip of the shell (Figs. 10, 11). In *G. d. granosissima*, however, the prismatic layer is visible only on the growth surface of the granulated ribs; between the ribs the nacre comes to the edge of the shell (Fig. 14).

The outer prismatic layer

The outer prismatic layer of calcite is very thin, and its ultrastructure could not be observed in stained, polished sections examined with the SEM. Nevertheless, in sections stained with Feigl's solution and examined under the dissecting microscope, the calcite layer was clearly revealed as the unstained outer surface of the shell (Fig. 3).

Shells of *G. d. demissa* consistently contain a dark purple pigment primarily visible on the outside of the shell and, in fact, contained in the thin outer prismatic layer. The pigment is revealed after the periostracum has been removed by bleaching. *G. d. granosissima* shells rarely contain this pigmentation; and even when it occurs, the coloration is of low intensity compared with the northern subspecies. Pigments in bivalves are thought to be waste products of metabolism incorporated into the shell (Comfort, 1951). Therefore, environmental differences should influence pigment deposition. However, both subspecies have a similar habitat and, in some areas (*e.g.*, Duval County, Florida), are conspecific. In these areas *G. d. demissa* is still more pigmented, suggesting that the difference in coloration is due to genetic rather than environmental variation.

DISCUSSION

In general, temperate species of mytilids have shells composed of both calcite and aragonite, whereas tropical species have shells composed entirely of aragonite (Taylor *et al.*, 1969). In addition, the calcite in shells of the temperate species is confined to a thin outer prismatic layer. In *Mytilus*, Dodd (1963, 1964) has shown quantitative relationships between both the shell (beak) structure and the

FIGURE 14. Growth surface of *Geukensia d. granosissima* showing prismatic layer (p) on the underside of a granule; note that the nacreous layer (n) comes down to the edge of the shell on either side of the granule. Scale bar is 100 μ m.

relative amount of aragonite on the one hand and the mean annual temperature of the habitat on the other. Nevertheless, although *G. demissa* has a geographical range from Nova Scotia to South Florida, we observed no differences in gross pattern of shell deposition: the shell mineralogy, throughout the range, appears to be similar to other temperate species. All of the shells of both subspecies in this study have a thin outer calcite layer. Bøggild (1930) found that, in *Mytilus edulis*, as in *Geukensia demissa*, the pigment is deposited in the outer layer of the shell. This may be a general characteristic of temperate mytilid shells.

In the middle prismatic layer, the subspecific difference in the deposition of the prisms is seen in the external gross morphology and on the growth surface (Figs. 9-14). In *G. d. granosissima* the granules are built up along the ribs as the prisms are deposited. The growth surface shows the underside of a fully developed granule (Fig. 14). As the next granule begins, nacre is deposited over the preceding granule. Since there are no granules in the troughs between the ribs, the nacre is laid down nearly to the edge over the prismatic layer. In *G. d. demissa*, the ribs are smooth so that the deposition of the prismatic layer occurs evenly along the lip of the shell in the troughs as well as the ribs (Fig. 11). Nacre is then laid down uniformly over the prismatic layer.

The inner nacreous layer comprises the bulk of the shell in both subspecies. The formation of new nacre crystals is not fully understood. Wada (1961, 1970) proposed several mechanisms involving screw dislocations. The position of small seed crystals developing near the edges of the 001 face of the underlying crystals (*e.g.*, Fig. 4) indicates that new crystallite formation occurs as hypothesized in Figure 15. As the parent crystal expands laterally, an inclination or thickening occurs along the edge initiating a screw dislocation and consequently a new crystal.

Growth of the nacre crystals occurs laterally along the *a*- and *b*-axes and vertically along the *c*-axis (axes indicated in Fig. 15). Lateral expansion of the crystals occurs concomitantly in several layers (*e.g.*, Fig. 4; Wise and Hay, 1968) and continues until a solid sheet is formed (Wada, 1968). Vertical development of the crystals along the *c*-axis is limited, possibly by the formation of insoluble interlamellar matrix over the crystal (proposed by Watabe, 1965; Wada, 1968). Discontinuities in the matrix could allow screw dislocations to occur (Taylor *et al.*, 1969; Bevelander and Nakahara, 1969). In fact, we have seen screw dislocations arising along the growth surface as the shell thickens (Fig. 6).

The regular orientation of nacre crystals in localized regions of the shell is characteristic of most molluscs (Wilbur, 1972). In Figure 4, for example, the crystals are aligned so that their axes are roughly parallel. Several mechanisms (reviewed by Grégoire, 1972) have been suggested to explain the uniform arrangement. Exogenous factors, such as the direction of growth of the mantle, the fibrillar arrangement in the matrix, and the direction of extrapallial fluid currents, have been proposed to explain growth and orientation of the crystals. A combination of factors is probably responsible for crystal alignment. The function of the mantle in the formation of the matrix may also include the proper orientation of the matrix fibrils seen by Mutvei (1969) and Wada (1970). If crystal nucleation occurs by screw dislocation (Fig. 15), the crystallographic axes of the seed crystal would correspond to those of the parent crystal. The whole mechanism

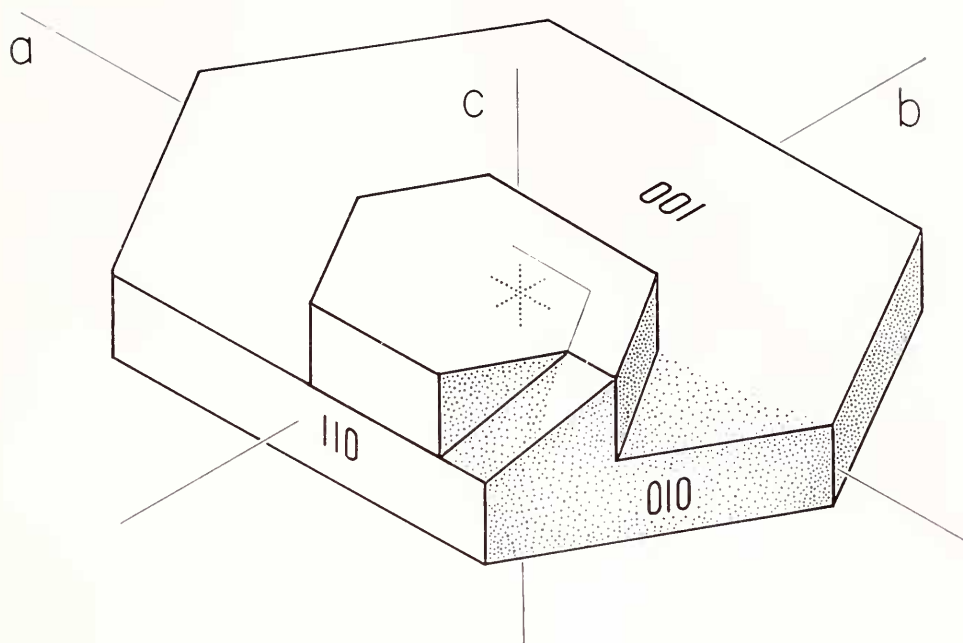


FIGURE 15. Three dimensional diagram of a screw dislocation in hexagonal nacre crystal with crystallographic axes: a, b, c; faces: 001, 110, 010. The insoluble matrix limits the growth along the c axis; discontinuities in the matrix allow the crystal to continue growing vertically and to form a new small seed crystal with axes and faces corresponding to those of the underlying crystal.

can be described as an interdependent relationship; the mantle directly influences the fibril direction in the matrix which, along with the already developed parent crystal, orients the axes of the seed crystals.

The gross differences in shell morphology seen in the two subspecies reflect the variation in deposition of the prismatic aragonite. The differences in ultrastructure cited here are a result of genetic rather than environmental influences.

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SUMMARY

1. The shells of two subspecies of the mussel, *Geukensia demissa*, were examined with the scanning electron microscope. In both subspecies, the shells contain an inner nacreous and middle and outer prismatic layers.

2. The inner nacreous layer is composed of laminae of contiguous, hexagonal, aragonite crystals.

3. The middle layer is composed of aragonite prisms arranged in a flabellate pattern.

4. Two subspecific differences in ultrastructure, reflecting the gross morphological subspecific variation in ribbing, were evident in the middle prismatic layer. First, in *G. d. granosissima*, which has narrow granulated ribs, the orientation of the aragonite prisms with respect to the nacre changes from 0° to 90° from one side to the other of each granule; *G. d. demissa* has no granules and the prisms are always perpendicular to the nacre.

Secondly, in *G. d. demissa*, the lip of the shell is a continuous band of the prismatic layer. In *G. d. granosissima*, however, the lip is primarily nacre interspersed with clusters of aragonite prisms corresponding to the underside of a granulated rib.

5. The subspecific difference in the deposition of prismatic aragonite is genetic rather than environmental.

6. The thin outer layer could only be observed after specific staining for calcite. This layer also contains the purple pigment found commonly in *G. d. demissa* but rarely in *G. d. granosissima*.

7. A model involving screw dislocation is presented to explain the regularity of nacre crystal orientation.

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